

O P I C

OFFICE DE LA PROPRIÉTÉ
INTELLECTUELLE DU CANADA



C I P O

CANADIAN INTELLECTUAL
PROPERTY OFFICE

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,200,794**
(22) 1997/03/24
(43) 1998/09/24

(72) ST. GEORGE-HYSLOP, Peter H., CA

(72) FRASER, Paul E., CA

(71) THE GOVERNING COUNCIL OF THE UNIVERSITY OF TORONTO,
CA

(51) Int.Cl.⁶ C07H 21/00, C12Q 1/68, G01N 33/53, C07K 14/47, C07K 16/18,
C12Q 1/00, A61K 48/00, A61K 38/17, A61K 35/12, A01K 67/027,
C12Q 1/02

(54) **GENE ET PROTEINE RELIES A LA MALADIE D'ALZHEIMER**

(54) **GENE AND PROTEIN RELATED TO ALZHEIMER'S DISEASE**

2200794

GENE AND PROTEIN RELATED TO ALZHEIMER'S DISEASE

Field of the Invention

The present invention relates to Alzheimer's Disease and, more particularly, to the identification of a gene and gene product associated with Alzheimer's Disease.

Background of the Invention

In order to facilitate reference to various journal articles, a listing of articles is provided at the end of the specification.

10 *Wingless 1* (*Wnt1*) is a gene involved in signal transduction in *D. melanogaster*, *Xenopus*, rodents, human and many other vertebrates and invertebrates. The gene is involved in signaling during embryonic axis development, but it is also expressed in adult tissues.

15 *Wnt1* exerts its effect through binding to the receptor *Drosophila frizzled 2* (*Dfz2*)¹, modulating the activity of *disheveled*², which suppresses the activity of the cytoplasmic serine/threonine kinase *zeste white 3* (also known as glycogen synthase kinase 3b- GSK3b)³. Inhibition

20 of the phosphorylation of cytoplasmic proteins such as *armadillo* (also known as β -catenin) by inhibition of GSK3 β causes the accumulation of these proteins (normal phosphorylated arm proteins are targeted for rapid proteasome mediated degradation)^{3,4}.

25 Description of the Invention

Genetic linkage studies have defined that a subset of pedigrees segregating familial Alzheimer Disease (FAD) but lacking mutations in the β -amyloid precursor protein gene (β APP), presenilin 1 or presenilin 2 genes (PS2),

30 show cosegregation of the disease trait with genetic markers in the pericentromeric region of chromosome 12. The genetic markers D12S1057 and D12S1042 generate lod scores of $z \geq +3.00$ at low recombination fractions and significant evidence for co-inheritance with an FAD trait

35 using non-parametric statistics such as Affected Pedigree Member methods (M. Pericak-Vance, 11th International

Several candidate genes exist in the vicinity of these genetic marker loci, including the human *Wingless 1* gene (*hWnt1*) whose biochemical properties would place it in biochemical pathways involving other FAD
5 susceptibility genes such as β APP and PS1.

A role for the *Wnt* pathway in Alzheimer's Disease (AD) is revealed by two observations. First, GSK3 β is known to be the enzyme principally responsible for the hyperphosphorylation of the microtubule associated
10 protein Tau in AD brain tissue ⁵. Hyperphosphorylated Tau is in turn a principal component of the paired helical filament structures which form the Neurofibrillary Tangle, one of the major neuropathologic hallmarks of AD
⁶. Secondly, another member of the *armadillo* protein
15 family (GT24) has been shown to interact with PS1 (Levesque et al., in press). These observations suggest that mutations in the *hWnt1* gene may be responsible for some cases of AD. These mutations might cause aberrant regulation of intracellular functions mediated by GSK3b
20 (e.g. defective activation of the *Wnt* pathway causing suppression of GSK3 β mediated hyperphosphorylation of Tau) or GT24 (e.g. aberrant regulation of the interaction of GT24 with PS1 and PS2 with resultant defects in the known biological activities of PS1 and PS2 such as
25 abnormal sensitivity to apoptosis ⁷, aberrant processing of β APP ⁸ and aberrant processing of the proteasome subunit S5a (Fraser et al., In Press). Evidence for a possible defect in GSK3 β action in AD has been provided by biochemical and immunochemical assays showing
30 increased immunoreactivity in AD brain tissue where it is expressed in appropriate cell types ^{5,9}. Other deleterious effects of persistent activation of GSK3 β (also known as Tau Protein Kinase I - TPKI) include defects in energy metabolism and defects in choline acetyl transferase
35 activity (which is another biochemical marker of AD) ⁵.

The fact that *Wnt1* is predominantly expressed in

severe embryonic defects in axial patterning does not preclude a role for mutations other than homozygous null mutants (i.e. knockouts) in adult onset neurodegeneration. Missense mutations in other embryonic
5 signal transduction proteins important in axial patterning (i.e. Notch 3) have been associated with other adult onset neurodegenerative diseases such as CADASIL¹⁰.

The genomic DNA sequence of the human Wnt1 gene has been determined by van Ooyen et al., (23) and is shown in
10 Table 1; the deduced amino acid sequence is shown in Table 2.

Primer sequences have been prepared, as described in Example 1, which permit the PCR amplification of each exon of the hWnt1 gene, allowing examination of the
15 nucleotide sequence of the exons of this gene in a selected subject such as an AD patient. Amplified exons can be sequenced using standard methods and primers as described in Example 1.

Genomic DNA from white blood cells of an FAD patient
20 from the Tor 117 pedigree, associated with autopsy-proven FAD, was examined. A nucleotide substitution was found at nucleotide 1441 (nucleotide numbering as Table 1).

The T→A substitution in Exon 2 of the hWnt1 gene will lead to a non-conservative amino acid change, Ser88
25 being replaced by Arg.

The mutation co-segregates in the Tor 117 pedigree and is seen in four other AD affected members of the pedigree.

The identification of the association between FAD
30 and the hWnt1 gene by the present inventors enables numerous applications.

In one series of embodiments, this invention provides primers complementary to the Wnt1 gene sequence which may be used to identify mutations causing AD, as
35 exemplified by the identification of the missense mutation described in Example 1.

In accordance with another aspect of the invention, a recombinant vector for transforming a mammalian or invertebrate tissue cell to express a normal or mutant Wnt1 sequence in the cells is provided.

5 In another series of embodiments, the present invention provides for host cells which have been transfected or otherwise transformed with the nucleotide sequence of the Wnt1 gene. The cells may be transformed merely for purposes of propagating an inserted nucleic
10 acid construct, or may be transformed so as to express the normal or mutant Wnt1 protein. The transformed cells of the invention may be used in assays to identify proteins and/or other compounds which affect normal or mutant Wnt1 expression, which interact with the normal or
15 mutant Wnt1 proteins, and/or which modulate the function or effects of the normal or mutant proteins, or to produce Wnt1 proteins, fusion proteins, functional domains, antigenic determinants, and/or antibodies to the Wnt1 protein.

20 Transformed cells may also be implanted into hosts, including humans, for therapeutic or other reasons. Preferred host cells include mammalian cells from neuronal, fibroblast, bone marrow, spleen, organotypic or mixed cell cultures, as well as bacterial, yeast,
25 nematode, insect and other invertebrate cells. For uses as described below, preferred cells also include embryonic stem cells, zygotes, gametes, and germ line cells.

In another series of embodiments, the present
30 invention provides transgenic animal models for AD and other diseases or disorders associated with mutations in the Wnt1 gene. The animal may be essentially any mammal, including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates.
35 In addition, invertebrate models, including nematodes and insects, may be used for certain applications. The

including microinjection, transfection, or other forms of transformation of embryonic stem cells, zygotes, gametes, and germ line cells with vectors including genomic or cDNA fragments, minigenes, homologous recombination
5 vectors, viral insertion vectors and the like. Suitable vectors include vaccinia virus, adenovirus, adeno associated virus, retrovirus, liposome transport, neuraltropic viruses, and Herpes simplex virus. The animal models may include transgenic sequences comprising
10 or derived from the Wnt1 gene, including normal and mutant sequences, intronic, exonic and untranslated sequences, and sequences encoding subsets of the Wnt1 protein, such as functional domains. The major types of animal models provided include: (1) Animals in which a
15 normal human Wnt1 gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and animals in which a normal
20 human Wnt1 gene has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Wnt1 genes have been recombinantly "humanized"
25 by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting. (2) Animals in which a mutant human Wnt1 gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of
30 either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and animals in which a mutant human Wnt1 gene has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination
35 or gene targeting; and/or in which one or both copies of one of the animal's homologous Wnt1 genes have been

sequences encoding a mutant human homologue by homologous recombination or gene targeting. (3) Animals in which a mutant version of one of that animal's Wnt1 genes has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and/or in which a mutant version of one of that animal's Wnt1 genes has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination or gene targeting. (4) "Knock-out" animals in which one or both copies of one of the animal's Wnt1 genes have been partially or completely deleted by homologous recombination or gene targeting, or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences. In preferred embodiments, a transgenic mouse model for AD has a transgene encoding a normal human Wnt1 protein, a mutant human or murine Wnt1 protein, or a humanized normal or mutant murine Wnt1 protein.

The present invention also specifically provides for mutant or disease-causing variants of the human Wnt1 protein by disclosing a specific mutant sequences and by providing methods by which other such variants may be routinely obtained. Because the Wnt1 proteins may be used in a variety of diagnostic, therapeutic and recombinant applications, various subsets of the Wnt1 protein sequence and combinations of the Wnt1 protein sequence with heterologous sequences are also provided. For example, for use as immunogens or in binding assays, subsets of the Wnt1 protein sequence, including both normal and mutant sequences, are provided. Such protein sequences may comprise a small number of consecutive amino acid residues from the sequence disclosed herein but preferably include at least 4-8, and preferably at least 9-15 consecutive amino acid residues from a Wnt1

sequence include those corresponding to one or more of the functional domains or antigenic determinants of the Wnt1 protein and, in particular, may include either normal (wild-type) or mutant sequences. The invention
5 also provides for various protein constructs in which Wnt1 sequence, either complete or subsets, is joined to exogenous sequences to form fusion proteins and the like.

In another series of embodiments, the present invention provides for the production and use of
10 polyclonal and monoclonal antibodies, including antibody fragments, including Fab fragments, $F(ab')_2$, and single chain antibody fragments, which selectively bind to the Wnt1 protein, or to specific antigenic determinants of the protein for use in various diagnostic, therapeutic
15 and technical applications described herein. The antibodies may be raised in mouse, rabbit, goat or other suitable animals, or may be produced recombinantly in cultured cells such as hybridoma cell lines. Preferably, the antibodies are raised against Wnt1 sequences
20 comprising at least 4-8, and preferably at least 9-15 consecutive amino acid residues from the Wnt1 sequence.

In another series of embodiments, the present invention provides methods of screening or identifying proteins, small molecules or other compounds which are
25 capable of inducing or inhibiting the expression of the hWnt1 gene. The assays may be performed in vitro using non-transformed cells, immortalized cell lines, or recombinant cell lines, or in vivo using the transgenic animal models enabled herein. In particular, the assays
30 may detect the presence of increased or decreased expression of hWnt1 or other Wnt1-related genes or proteins on the basis of increased or decreased mRNA expression, increased or decreased levels of presenilin-related protein products; or increased or decreased
35 levels of expression of a marker gene (e.g., β -galactosidase, green fluorescent protein, alkaline

regulatory region in a recombinant construct. Cells known to express a particular Wnt1, or transformed to express Wnt1, are incubated and one or more test compounds are added to the medium. After allowing a sufficient period of time (e.g., 0-72 hours) for the compound to induce or inhibit the expression of the *hWnt1* gene, any change in levels of expression from an established baseline may be detected using any of the techniques described above. In particularly preferred embodiments, the cells are from an immortalized cell line such as a human neuroblastoma, glioblastoma or a hybridoma cell line, or are transformed cells of the invention.

In another series of embodiments, the present invention provides methods for identifying proteins and other compounds which bind to, or otherwise directly interact with, hWnt1 protein. The proteins and compounds will include endogenous cellular components which interact with the Wnt1 protein in vivo and which, therefore, provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic and otherwise exogenous compounds which may have Wnt1 protein binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one series of embodiments, cell lysates or tissue homogenates (e.g., human brain homogenates, lymphocyte lysates) may be screened for proteins or other compounds which bind either to normal or to mutant Wnt1 protein. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (e.g., libraries of small molecules or peptides), may be screened for Wnt1 binding capacity. In each of these embodiments, an assay is conducted to detect binding between a "Wnt1 component" and some other moiety. The "Wnt1 component" in these assays may be any polypeptide comprising or derived from a normal or mutant Wnt1 protein, including functional domains or antigenic

proteins. Binding may be detected by non-specific measures (e.g., changes in intracellular Ca^{2+} , GTP/GDP ratio) or by specific measures (e.g., changes in Ab peptide production or changes in the expression of other downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods). The preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of Wnt1 components and bound proteins or other compounds by immunoprecipitation; (3) the Biomolecular Interaction Assay (BIAcore); and (4) the yeast two-hybrid systems.

The identification of other cellular proteins which interact with the hWnt1 protein, as described above, enables the identification of other genes involved in the biochemical pathway causing AD.

Alternatively, tissues, fluids or cells of AD-affected or at-risk subjects can be used for the analysis of the DNA sequence, transcriptional pattern, protein expression, protein post-translational modification (phosphorylation etc), and biochemical/functional activity of other genes known to function in the same biochemical pathways as these genes. Thus, for Wnt1, these other genes would include other members of the mammalian *Wingless* family of genes, other genes in the known *Wingless* signalling pathways (e.g. *frizzled* receptors, *dishevelled* homologues, glycogen synthetase kinase 3 β , other armadillo proteins), and other genes known to be involved in process of wingless (e.g. mammalian homologues of *porcupine*¹¹).

In another series of embodiments, the present invention provides for methods of identifying proteins, small molecules and other compounds capable of modulating the activity of normal or mutant Wnt1 protein. Using normal cells or animals, the transformed cells and

cells obtained from subjects bearing normal or mutant *hWnt1* genes, the present invention provides methods of identifying such compounds on the basis of their ability to affect the expression of the *Wnt1*, the intracellular localization of *Wnt1* protein, intracellular Ca^{2+} , Na^{+} , K^{+} or other ion levels or metabolism, the occurrence or rate of apoptosis or cell death, the levels or pattern of Ab peptide production, the presence or levels of phosphorylation of microtubule associated proteins, or other biochemical, histological, or physiological markers which distinguish cells bearing normal and mutant *Wnt1* sequences. Using the transgenic animals of the invention, methods of identifying such compounds are also provided on the basis of the ability of the compounds to affect behavioral, physiological or histological phenotypes associated with mutations in *Wnt1* protein.

In another series of embodiments, the present invention provides methods for screening for carriers of *Wnt1* alleles associated with AD, for diagnosis of victims of AD, and for the screening and diagnosis of related presenile and senile dementias, psychiatric diseases such as schizophrenia and depression, and neurologic diseases such as stroke and cerebral hemorrhage, which associated with mutations in the *Wnt1* gene. Screening and/or diagnosis can be accomplished by methods based upon the nucleic acids, proteins, and/or antibodies disclosed and enabled herein, including functional assays designed to detect failure or augmentation of the normal *Wnt1* activity and/or the presence of specific new activities conferred by the mutant *Wnt1* protein. Thus, screens and diagnostics based upon *Wnt1* proteins are provided which detect differences between mutant and normal *Wnt1* in electrophoretic mobility, in proteolytic cleavage patterns, in molar ratios of the various amino acid residues, in ability to bind specific antibodies. In addition, screens and diagnostics based upon nucleic

in nucleotide sequences by direct nucleotide sequencing, hybridization using allele specific oligonucleotides, restriction enzyme digest and mapping (e.g., RFLP, REF-SSCP), electrophoretic mobility (e.g., SSCP, DGGE), PCR mapping, RNase protection, chemical mismatch cleavage, ligase-mediated detection, and various other methods. In accordance with these embodiments, diagnostic kits are also provided which will include the reagents necessary for the above-described diagnostic screens.

10 In another series of embodiments, the present invention provides methods and pharmaceutical preparations for use in the treatment of Wnt1-associated diseases such as AD. These methods and pharmaceuticals are based upon (1) administration of normal Wnt1 proteins, (2) gene therapy with a normal Wnt1 gene to 15 compensate for or replace a mutant gene, (3) gene therapy based upon antisense sequences to a mutant Wnt1 gene or which "knock-out" the mutant gene, (4) gene therapy based upon sequences which encode a protein which blocks or 20 corrects the deleterious effects of Wnt1 mutants, (5) immunotherapy based upon antibodies to normal and/or mutant Wnt1 proteins, or (6) small molecules (drugs) which alter Wnt1 expression, block abnormal interactions between mutant forms of Wnt1 and other proteins or 25 ligands, or which otherwise block the aberrant function of mutant Wnt1 proteins by altering the structure of the mutant proteins, by enhancing their metabolic clearance, or by inhibiting their function.

The activity of *hWnt1* can be manipulated 30 pharmacologically by several mechanisms including drugs which promote or inhibit post-translational modification of the Wnt1 protein (e.g. phorbol esters okadaic acid to alter phosphorylation state, inhibitors of glycosylation etc.).

35 The wingless signalling pathway is well worked out

D. melanogaster, Xenopus, rodents and many other

invertebrate and vertebrate animals. These systems can be used to devise means of altering the activity of mutant or wild type wingless signalling pathways (ie wingless and its downstream partners including glycogen synthase kinase 3 beta) in humans with AD regardless of whether AD arises as a result of mutations in wingless or arising through other mechanisms. Such modulating mechanisms would include the following items.

Wnt signalling pathways can be modulated by Lithium (24), by protein kinase C inhibitors such as Ro31-8220 as well as phorbol esters (25), as well as by decapentaplegic protein (24) and Notch (27) either by directly influencing Wingless expression or by influencing downstream elements in the wingless signaling pathway. These, or analogues or antagonists could be used to modulate wingless signaling (and relevant downstream events such as glycogen synthase kinase 3-beta (GSK-3b) activation in patients with AD and can be used as starting points for drug design.

Because *Wnt1* binds to specific proteins and/or receptor-like molecules (*Wnt1* binds to *fizzled* receptors¹) these interactions can be modelled and used to develop selective antagonists, agonist, competitive inhibitors or competitive agonists which possess selective activities against mutant isoforms of these proteins.

The sequences can be used to identify other genes involved in the biochemical pathway causing AD by employing techniques such as yeast-two-hybrid methods to identify other cellular proteins interacting with the respective protein. Alternatively, tissues, fluids or cells of AD affected or at-risk subjects can be used for the analysis of the DNA sequence, transcriptional pattern, protein expression, protein post-translational modification (phosphorylation etc), and

function in the same biochemical pathways as these genes. Thus, for *Wnt1* these other genes would include other members of the mammalian *Wingless* family of genes, other genes in the known *Wingless* signaling pathways
5 (e.g. *frizzled* receptors, *dishevelled* homologues, glycogen synthetase kinase 3 β , other armadillo proteins), and other genes known to be involved in processing of wingless (e.g. mammalian homologues of *porcupine*¹¹).

The nucleotide sequences and/or proteins can
10 themselves be used as direct therapeutic agents. Dominant loss of function mutations (constitutively inactive) and dominant gain of function mutations (constitutively active) have been described in *Wnt1*. Thus if disease causing mutations in these genes have a
15 dominant negative effect, appropriate nucleotides or recombinant proteins can be made with a countervailing dominant gain of function activities and administered via several routes such as protein infusion (*Wnt1* is a soluble extracellular molecule) or transfection (using
20 vectors such as H. simplex).

Assays exist for the *Wnt1* gene (e.g. transformation of C57MG mammary epithelial cell lines¹², myogenesis in somites and segmental plates co-cultured with *Wnt1*¹³, biochemical assays of *armadillo* levels in cultured
25 *Drosophila* cl-8 imaginal disc cell lines¹⁴). These *in vitro* assays can be used to judge the effect of the FAD related missense mutations and to screen for drugs which act at *Wnt1* itself, at upstream, or at downstream sites in the *Wnt* signaling pathways, and which might be used to
30 counteract the effect of the *Wnt1* mutations.

In accordance with another aspect of the invention, the proteins of the invention can be used as starting points for rational drug design to provide ligands, therapeutic drugs or other types of small chemical

compounds identified by screening assays may serve as "lead compounds" in rational drug design.

Example 1

Genomic sequences for each exon of *hWnt1* can be amplified
5 using primers 1306 (5'-AGCCTCCTCCCGTCACTTCAG) and 1307
(5'-GGATCATTCGCCCCACTTGTA) for Exon 1; 1308 (5'-
CTGGGAGAGCGGGTATTATTA) and 1309 (5'-CTGGGCACGAGGCACTTGG)
for Exon 2: and 1310 (5'-CTGCTCCACTTCCGCTATCG) and 1311
(5'-TGCCCCTTGCCTTATCTCAC) for Exon 3; and 1312 (5'-
10 CCTGAGAGGCCGAGACTGACT) and 1313 (5'-
GGAGAGATGGGATGCGTATGAA) for Exon 4. PCR conditions for
these primers are Exon 1: 100ng genomic DNA, MgCl₂ 1.5 mM,
dNTPs 250 mM, primers 50 pmol, Taq polymerase 0.5 Units,
DMSO 5% in reaction volume of 50 ul with thermocycles of
15 94°C X 30secs, 58°C X 20secs, 72°C X 30secs, for 34
cycles. Exon 2: as for Exon 1 except MgCl₂ 2.5 mM, DMSO
10% and annealing temperature of 60°C. Exon 3: as for Exon
1 except MgCl₂ 2.5 mM, no DMSO, annealing temperature of
58°C. Exon 4: as for Exon 1 except MgCl₂ 1.5 mM, DMSO 5%,
20 and annealing temperature of 59°C.

These exons can then be sequenced using standard
dideoxy-cycle sequencing methods employing primers 1316
(5'-CGGGCAACAACCAAAGTC) for Exon 1; 1317 (5'-
CGGGTGGCACAGTTTTTA) for Exon 2; 1318 (5'-
25 CCCCTTGCCTTATCTCAC) for Exon 3; 1319 (5'-
CCGGGAGAGGGCAGTGTC) and 1327 (5'-AACCGGGTCTTGAGTGCT) for
Exon 4.

Analysis of the genomic sequences for Exons 1-4 of
hWnt1 detected a T→A substitution at nucleotide 1441
30 (Figure 1: Accession #:X03072) in Exon 2 coding sequence
which results in the non-conservative substitution of
Ser88 by Arg (*hWnt1* Ser88Arg) in an affected member of a
pedigree (Tor117) segregating autopsy-proven Familial

2200794

15

in vertebrates above birds and in *Xenopus*, and is conservatively substituted by Ala or Thr in some lower vertebrates, and by Ala, Leu, Iso, or Thr in most other *Wnt* homologues such as *Wnt3*, *Wnt3a*, *Wnt5*, *Wnt5a*.

2200794

16

Table 1

Accession # X03072 (G DB genome)

ORIGIN

```
1   cagctgagtg aggcgggagc gcgtgggagg gtgtcccaag gggagggggtc cgcggccagt
5 61   gcaggccccg aggcgggggc caccgggcag ggggcggggg tgagccccga cggccaaccc
121  gtcagctctc ggctcagacg ggcgggaacc acagccccgc tcgctgcca ttgtctgcgc
181  ccctaaccgg tgcgccctgg tgccacagtg cggccccgag gggcagcctc ctcccgtcac
241  ttcagccagc gccgcaacta taagaggcgg tgccgccccg cgtggccgcc tcagcccacc
301  agccgggacc gcgagccatg ctgtccgcgc cccgccccca gggttgttaa agccagactg
10 361  cgaactctcg cactgcccgc caccgcgcgc tcccgtccca ccgtcgcggg caacaaccaa
421  agtcgccgca actgcagcac agagcgggca aagccaggca ggccatgggg ctctgggcgc
481  tggtgcctgg ctgggtttct gctacgctgc tgctggcgct ggccgctctg cccgcagccc
541  tggctgcaa cagcagtggc cgatggtggt aagtgcgctg gtgcgggggtc gccacttgtc
601  ccgcggcaca gagccagggg ccaaccctac ccagctccca cgctctggga tccgtctgca
15 661  gacaggctcc ctccccgctc tgacttcctt ccgcgacacc gaagggcgat ctggcatgaa
721  actgccccag actccagctc tgtacaagtg gggcgaatga tccgcccgcg gaggcctaag
781  ataccccagg caggagccc actctcatct agcaccgccc ttccccttg agcgccaaact
841  ccagcctcac ggcggtggct caccacaggt ttccccacct cgggaagtga agggccagga
901  gttcgcctag aaaggagggg agaagagggt gggactccta agcatttcac gccttgggtg
20 961  ggcaagaact gcaggccatg attatctcgc tcaggctgac cggaagaggc tcggagatcc
1021 aaggtagaca ctcggtctcc gggtagctcc tctgtccagt ctccggacct agggctcagg
1081 cgagcagccc tgggactact gggcacacac aagtctggac gccagttct tcaaaattag
1141 tgagcctggg agagcgggta ttattaatct cccgccatto tctccagcca cataccccca
1201 ggaagaggac cgggtggcac agtttttatg gttagggtgc ggatcccctt cctgagcctg
25 1261 agctatcata cgtcccacca ggggtattgt gaacgtagcc tcctccacga acctgcttac
1321 agactccaag agtctgcaac tggtagctga gccagctctg cagctgttga gccgcaaaca
1381 gcggcgcctg atacgcaaaa atccggggat cctgcacagc gtgagtgggg ggctgcagag
1441 tgccgtgcgc gagtgcaggt ggcagttccg gaatcgccgc tggaactgtc ccactgctcc
1501 agggccccac ctcttcggca agatcgtcaa ccgaggtggg tgcccaggaa ggcgacgctt
30 1561 ccgggagcag gggaaacgcg gggtcacccc cagggcattg gcgggagagt tcagagaagg
1621 tgtcccaggc gcctggaggg tcacacaatc aaccttgcca agtgccctgt gccagcgcc
1681 agctcggggc cagacttcta ccaggcgttt tccagccgtg caccctggaa acgaagctta
1741 acttttctga gctactgcc cagataaaga aagtttcggg tcgcggacgc cggctgaccg
1801 ccgctttccc ccagcctctc taaaagcgc ctgggaagct gctctctgca ggcgtgtgtc
35 1861 tggcctctcg cccagcaagg cttgcaccgc caaatgggc cgaaagtttt gggctgcgaa
```

2200794

17

Table 1 Continued

2041	gcccgtggac	gtggctgcgt	gccacgcac	ctgctttctc	tactagccct	agagaccagc
2101	tttccagcac	tgccggccct	ggcctcagg	actcaaagtg	cggagtcggg	ggtgggattc
2161	cggteccaag	cccttcatga	gggtgctggc	cgcgccccgc	gtacccccctc	gctgatcccc
5 2221	gctcccttct	cccacaggct	gtcgagaaac	ggcgtttatc	ttcgctatca	cctccgccgg
2281	ggtcaccat	tcggtggcgc	gctcctgctc	agaaggttcc	atcgaatcct	gcacgtgtga
2341	ctaccggcgg	cgcggccccg	ggggccccga	ctggcactgg	gggggctgca	gcgacaacat
2401	tgacttcggc	cgcctcttcg	gccgggagtt	cgtggactcc	ggggagaagg	ggcgggacct
2461	gcgcttcctc	atgaaccttc	acaacaacga	ggcaggccgt	acggtgagct	ttgagaggct
10 2521	ccgcacccta	agcggagcgg	caggggcca	cctcgggctg	gggaagtga	ggtcgggtga
2581	ataaggcaag	gggcaccagg	agagggcgtc	ctgggagagc	cggaggcttg	gaacgaagac
2641	ggagaataga	ggagacagt	gctgagggca	aaggtatgtc	tggccccgcg	acaggtagaa
2701	gaggttgcaa	atcaagcaca	gtctcttcgc	tgtacagatt	cgaaaaataa	gcctgagagg
2761	ccgagactga	ctcgccgcgg	cggagcaggg	ttgggcaggg	tttccaaatc	tcagcggaa
15 2821	atttcgcgcc	tcccttcccc	tgggctcagc	taggcctggg	cctttgctga	ggtccggccc
2881	ccgtggcgct	cgggagaggg	cagtgtctgg	gaggggtgact	ctggccccgt	gccctgggac
2941	actctttctt	cccctatccc	cgcagaccgt	attctccgag	atgcgccagg	agtgaagtga
3001	ccacgggatg	tccggctcat	gcacgggtgc	cacgtgctgg	atgcggctgc	ccacgctgcg
3061	cgcggtgggc	gatgtgctgc	gcgaccgctt	cgacggcgcc	tcgcgcgtcc	tgtacggcaa
20 3121	ccgcggcagc	aaccgcgctt	cgcgagcgga	gctgctgcgc	ctggagccgg	aagaccgggc
3181	ccacaaaccg	ccctcccccc	acgacctcgt	ctacttcgag	aaatcgccca	acttctgcac
3241	gtacagcgga	cgcctgggca	cagcaggcac	ggcagggcgc	gcctgtaaca	gctcgtcgcc
3301	cgcgctggac	ggctgcgagc	tgctctgctg	cggcaggggc	caccgcacgc	gcacgcagcg
3361	cgtcaccgag	cgtgcaact	gcaccttcca	ctggtgctgc	cacgtcagct	gccgcaactg
25 3421	cacgcacacg	cgcgtactgc	acgagtgtct	gtgaggcgct	gcgcggactc	gccccagga
3481	acgtctctct	cgagccctcc	cccaaacaga	ctcgctagca	ctcaagacct	ggttattcgc
3541	ccacccgagt	acctccagtc	acactccccg	cggttcatac	gcatcccatc	tctcccactt
3601	cctcctacct	ggggactcct	caaaccactt	gcctggggcg	gcatgaacct	tcttgccatc
3661	ctgatggacc	tgccccggac	ctaacctccc	tccctctccg	cgggagacct	cttgttgac
30 3721	tgccccctgc	ttggccagga	ggtgagagaa	ggatgggtcc	cctccgccat	ggggtcggct
3781	cctgatgggt	tcattctgcc	tgctccatcg	cgccagcgac	ctctctgcct	ctcttcttcc
3841	cctttgtcct	gcgttttctc	cgggtcctcc	taagtccctt	cctattctcc	tgccatgggt
3901	gcagaccctg	aaccacacc	tgggcatcag	ggcctttctc	ctccccacct	gtagctgaag
3961	caggagggtta	cagggcaaaa	gggcagctgt	gatgatgtgg	gaatgagggt	gggggaacca
35 4021	gcagaaatgc	ccccattctc	ccagtctctg	tcgtggagcc	attgaacagc	tgtgagccat
4081	gcctccctgg	gccacctcct	acccttctct	gtcctgcctc	ctcatcagtg	tgtaataat

2200794

18

Table 1 Continued

4261 tcttctctcc tttctccctg cagccttttc tgggccctct tctctcctca gtttctcaaa
 4321 gatgcgtttg cctcctggaa tcagtatttc cttccactgt agctattagc ggctcctcgc
 4381 ccccaccagt gtagcatctt cctctgcaga ataaaatctc tattttttatc gatgacttgg
 5 4441 tggcttttcc ttgaatccag aacacaacct tgtttgtggt gtcccctatc ctcccctttt
 4501 accactccca gcttgggaagc tt

LOCUS HSINT1G 4522 bp DNA PRI 03-JAN-1991
 DEFINITION Human int-1 mammary oncogene.
 10 ACCESSION X03072
 NID g33935
 KEYWORDS int-1 oncogene; oncogene.
 SOURCE human.
 ORGANISM Homo sapiens
 15 Eukaryotae; mitochondrial eukaryotes; Metazoa;
 Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 4522)
 20 AUTHORS van Ooyen,A., Kwee,V. and Nusse,R.
 TITLE The nucleotide sequence of the human int-1
 mammary oncogene;
 evolutionary conservation of coding and non-
 coding sequences
 25 JOURNAL EMBO J. 4 (11), 2905-2909 (1985)
 MEDLINE 86055728
 COMMENT Data kindly reviewed (15-JUN-1986) by R. Nusse.
 FEATURES Location/Qualifiers
 source 1..4522
 30 /organism="Homo sapiens"
 promoter 259..263
 /note="pot. TATA-box"
 CDS join(465..568,1282..1535,2238..2503,2966..3454)
 /codon_start=1
 35 /product="int-1 protein"

2200794

19

Table 1 Continued

```

      /db_xref="SWISS-PROT:P04628"
intron 569..1281
5      /note="intron I"
intron 1536..2237
      /note="intron II"
intron 2504..2965
      /note="intron III"
10 misc_feature 4410..4415
      /note="pot. polyadenylation signal"
BASE COUNT      805 a   1523 c   1320 g   874 t
```

2200794

20

Table 2

MTSRSTARPNQPPQASKICQFKLVLLGESAVGKSSLVLRVKGQ
FHEYQESTIGAAFLTQSVCLDDTTVKFEIWDTAGQERYHSLAPMYRGAQAQAAIVVYDI
5 TNQETFARAKTWVKELQRQASPSIVIALAGNKADLANCRMVEYEEAQAYADDNSLLFM
ETSAKTAMNVNDLFLAIAKKLPKSEPQNLGGAAGRSRGVDLHEQSQQNKSQCCSN

References

1. Bhanot, P., Brink, N., Samos, C.H., Hsieh, J.-C.,
Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and
Nusse, R., A new member of the frizzled family from
5 *Drosophila* functions as a Wingless receptor., *Nature*,
1996. **382**: 225-230.
2. Klingensmith, J., Nusse, R., and Perrimon, N., The
Drosophila segment polarity gene *dishevelled* encodes a
novel protein required for response to the wingless
10 signal., *Genes & Develop.*, 1994. **8**: 118-130.
3. Seigfreid, E., Wilder, E.L., and Perrimon, N.,
Components of wingless signalling in *Drosophila*., *Nature*,
1994. **367**: 76-80.
4. Yost, C., Torres, M., Miller, J.R., Huang, E.,
15 Kimelman, D., and Moon, R.T., The axis-inducing activity,
stability, and subcellular distribution of B-catenin is
regulated in *Xenopus* embryos by glycogen synthetase
kinase 3., *Genes & Develop.*, 1996. **10**: 1443-1454.
5. Imahori, K. and Uchida, T., Physiology and pathology
20 of Tau protein kinases in relation to Alzheimer's
Disease., *J. Biochem.*, 1997. **121**: 179-188.
6. Kosik, K. and Greenberg, S.M., *Tau protein and
Alzheimer Disease.*, in *Alzheimer Disease.*, R.D. Terry, R.
Katzman, and K.L. Bick, Editor. 1994, Raven Press: New
25 York. 335-344.
7. Wolozin, B., Iwasaki, K., Vito, P., Ganjei, K.,
Lacana, E., Sunderland, T., Zhao, B., Kusiak, J.W., and
D'Adamio, L., Participation of Presenilin 2 in apoptosis:
enhanced basal activity conferred by an Alzheimer
30 Mutation., *Science*, 1996. **274**: 1710-1713.

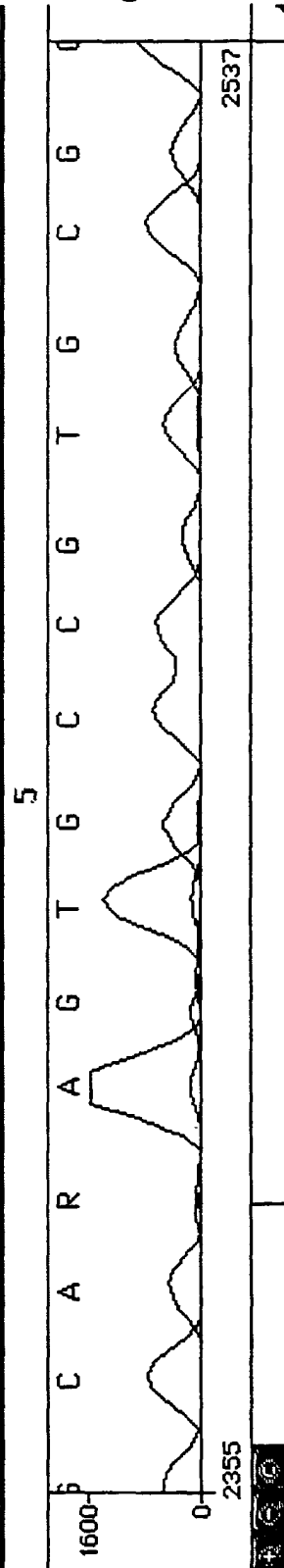
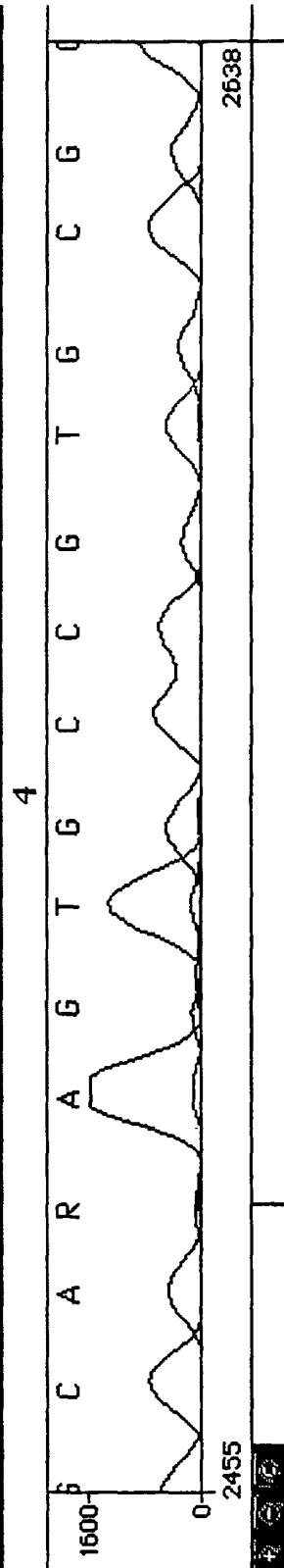
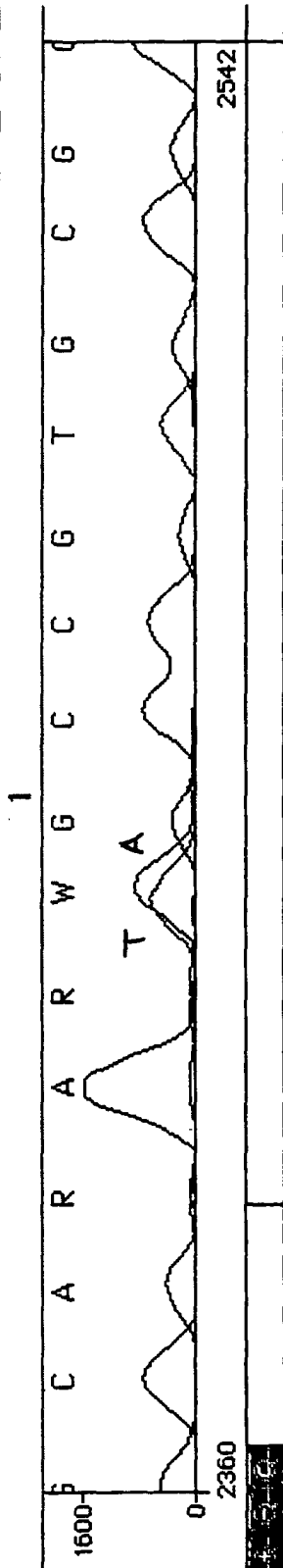
- 42 residue amyloid B-protein in both transfected cells and transgenic mice., *Nature Med.*, 1997. **in the press.**
9. Shiura, R.A., Ishiguro, K., Takahashi, M., et al., Immunocytochemistry of tau-phosphoserine-413 and tau
5 protein kinase 1 (GSK3b) in Alzheimer pathology., *Brain Res*, 1996. **737**: 119-132.
10. Joutel, A., Corpechot, C., Ducros, A., et al., Notch3 mutations in CADASIL, a hereditary adult-onset condition with stroke and dementia., *Nature*, 1996. **383**:
10 707-710.
11. Kadowaki, T., Wilder, E., Klingensmith, J., Zachary, K., and Perrimon, N., The segment polarity gene porcupine encodes a putative multitransmembrane protein involved in wingless processing., *Genes & Develop.*, 1996. **10**:
15 3116-3128.
12. Wong, G.T., Gavin, B.J., and McMahon, A.P., Differential transformation of mammary epithelial cells by wnt genes., *Mol. Cell Biol.*, 1994. **14**: 6278-6286.
13. Stern, H.M., Brown, A.M., and Hauschka, S.D.,
20 Myogenesis in paraxial mesoderm: preferential induction by dorsal neural tube and cells expressing Wnt1., *Development*, 1995. **121**: 3675-3686.
14. van Leeuwen, F., Samos, C.H., and Nusse, R., Biological activity of soluble wingless protein in
25 cultured *Drosophila* imaginal disc cells., *Nature*, 1994. **368**: 342-344.
15. Ullrich, O., Reinsch, S., Urbe, S., Zerial, M., and Parton, G., Rab11 regulates recycling through the pericentriolar recycling endosome., *J. Cell Biol.*, 1996.
30 **135**: 913-924.

16. Bucci, C., Lutcke, A., Steele-Mortimer, O., Olkkonen, V.M., Dupree, P., Chiariello, M., Bruni, C.B., Simons, K., and Zerial, M., Co-operative regulation of endocytosis by three RAB5 isoforms., FEBS Letters, 1995. 5 366: 65-71.
17. Harter, C. and Weiland, F., The secretory pathway: mechanisms of protein sorting and transport., Biochim. Biophys. Acta, 1996. 1286: 75-93.
18. Ikin, A., Annaert, W.G., Takei, K., Decamilli, P., 10 Jahn, R., Greengard, P., and Buxbaum, J.D., Amyloid protein precursor is localized in nerve terminal preparations to rab5-containing vesicular organelles distinct from those implicated in the synaptic vesicle pathway., J. Biol. Chem., 1996. 271: 31783-31786.
- 15 19. McConlogue, L., Castellano, F., de Wit, C., Schenk, D., and Maltese, W.A., Differential effects of Rab6 mutant on secretory versus amyloidogenic processing of Alzheimer's B-amyloid precursor protein., J. Biol.Chem., 1996. 271: 1343-1348.
- 20 20. Dugan, J.M., de Wit, C., McConlogue, L., and Maltese, W.A., The RAS-related GTP-binding protein, Rab1b, regulates early steps in exocytic transport and processing of beta-amyloid precursor protein., J.Biol.Chem., 1995. 270: 10982-10989.
- 25 21. Stenmark, H., Vitale, G., Ullrich, O., and Zerial, M., Rabaptin-5 is a direct effector of the small GTPase RAB5 in endocytic membrane fusion., Cell, 1995. 83: 423-433.
22. Hoffenberg, S., Sanford, J.C., Liu, S.B., Daniel, 30 D.S., Tuvin, M., Knoll, B.J., Wesslingresnick, M., and Dickey, B.F., Biochemical and functional characterization of a recombinant GTPase, RAB5, and two of its mutants.,

23. Van Ooyen, A., Kuree, V. and Nusse, R. (1985) *EMBO J.*, **4**, 2905-2909.
24. Stambolic V, Ruel, L. Woodgett, J.R. Lithium inhibits glycogen synthetase kinase 3 activity and mimics wingless signalling in intact cells. *Current Biology* 6, 1664-1668. 1996
25. Cook, D., Fry, M.J., Hughes, K., Sumathipala, R., Woodgett, J.R., Dale, T.C. Wingless inactivates glycogen-synthase kinase 3 via an intracellular signalling pathway which involves protein kinase c. *EMBO Journal* 15; 4526-4536, 1996.
26. Brook, W.J. Cohen, S.M. antagonistic interactions between wingless and decapentaplegic responsible for dorsal-ventral pattern in the *Drosophila* leg. *Science* 273: 1373-1377, 1996.
27. Rulifson, E.J. Blair, SS Notch regulates wingless expression and is not required for reception of the paracrine wingless signal during margin neurogenesis in *Drosophila*. *Development* 121 2813-2824, 1995.

EH n2Mar7,97P1317.2

1 6967 GTGAGTGGG GGTGCGAR WCCGTGGC GAGTCAAGT GGCAGTCCG GAATCGCCG TGGAACTGTC
 2 6968 GTGAGTGGG GGTGCGARAG TGCCGTGGC GAGTCAAGT GGCAGTCCG GAATCGCCG TGGAACTGTC
 3 6969 GTGAGTGGG GGTGCGAR TGCCGTGGC GAGTCAAGT GGCAGTCCG GAATCGCCG TGGAACTGTC
 4 6970 GTGAGTGGG GGTGCGAR TGCCGTGGC GAGTCAAGT GGCAGTCCG GAATCGCCG TGGAACTGTC
 5 6971 GTGAGTGGG GGTGCGARAG TGCCGTGGC GAGTCAAGT GGCAGTCCG GAATCGCCG TGGAACTGTC



Affected
Member
Tot 117

2200794

Control

FIGURE 1